Operating Manual

Capillary Running Module

MINI (2811.1) & MAXI (2816.1)

WARNING:
Please read the entire operator's manual thoroughly before operating this unit.

Warning:
Like all apparatus run by electricity these units are capable of delivering potentially lethal voltage when connected to a power supply. They should be operated only by qualified technically trained personnel. The capillary electrophoresis modules from ROTH are designed for long term laboratory use and to obtain reproducible results. Please spend a few moments reading the instruction manual thoroughly.

These units comply with the statutory CE safety rules:
73/23/EEC: Low voltage directive: IEC 1010-1:1990 plus amendment 1:1992
EN 61010-1:1993/BS EN 61010-1:1993

Please verify, that you received the unit completely and without any damage. Any faults or losses have to be reported to ROTH immediately. ROTH can not accept responsibility for goods, that were sent back without informing them.

Please retain all packaging material until the warranty period has expired.
For further information, please contact us at Tel.: 0721/5606-0.

SPECIFICATIONS:

Technical features:
- ✓ Durable acrylic construction
- ✓ All acrylic joints chemically bonded
- ✓ Doubly insulated cables, rated safe up to 1.000 volts
- ✓ Gold plated electrical connectors, corrosion-free and rated safe up to 1.000 volts
- ✓ Recessed power connectors, integral with the safety lid
- ✓ 0.2 mm diameter platinum electrodes, 99.99 % pure
- ✓ User replacable platinum electrodes

Environmental Conditions

- ✓ This apparatus is intended for indoor use only.
- ✓ The unit can be operated safely at an altitude of .2000 m.
- ✓ The normal operating temperature range is between 4 °C and 65 °C.
- ✓ Maximum relative humidity 80 % for temperatures up to 31 °C decreasing linearly to 50 % relative humidity at 40 °C.

All Roth products available for delivery have undergone rigorous quality controls.

AVAILABLE ACCESSORIES:

(All accessories can be purchased from Carl Roth GmbH + Co. KG. Please use the indicated ordering numbers.)

Tank (without lid)		Y005.1
Cooled tank (without lid)		Y006.1
Replacement lid for tank		Y009.1
Running module		Y007.1
Casting module		Y008.1
U-shaped fixing plate + seals for running module (8 per set)		Y010.1
Seals for running module (8 per set)		Y011.1
Gaskets for casting module (2 per set)		T796.1
Replacement screws for running module (4 per set)		Y012.1
Replacement platin electrode (Ø 0,2 mm)		T794.1
Blanking Ports (silicone)	10 pieces	2815.1
Glass capillaries MINI, 8 cm length, 1 mm internal diameter	10 pieces	2812.1
Glass capillaries MINI, 8 cm length, 4 mm internal diameter	10 pieces	2813.1
Glass capillaries MAXI, 17 cm length, 1 mm internal diameter	10 pieces	2817.1
Glass capillaries MAXI, 17 cm length, 4 mm internal diameter	10 pieces	2818.1
Gel extraction platform MINI (for max. 8 cm gels)	1 piece	2814.1
Gel extraction platform MAXI (for max. 17 cm gels)	1 piece	2819.1

Tab. 1 Operational

Unit	**Max. voltage (V)**	**Max.current per gel (mA)**
MINI	1000 V	110 mA
MAXI		

Tab. 2 Technical Specifications

Units		**MINI**			**MAXI**	
Capillary Dimensions	Inner Diameter 1 mm	Outer Diameter 5 mm	Length 80 mm	Inner Diameter 1 mm	Outer Diameter 5 mm	Length 170 mm
Recommended Buffer Volume	Upper Buffer Chamber 100 ml		Gel Tank 600 ml	Upper Buffer Chamber 200 ml		Gel Tank 1.600 ml
Recommended Running Conditions for IEF or Capillary Gels	Prerun	Separation	End of Run	Prerun	Separation	End of Run
	200 V	400 V	500 V	200 V	400 V	500 V
	30 min	3 h	30 min	30 min	6 - 18 h	30 min
	100 Vh	1.200 Vh	250 Vh	100 Vh	2.400 Vh	250 Vh

USING THE CAPILLARY GEL ELECTROPHORESIS UNITS

A. Safety Precautions

Please read the entire instruction manual thoroughly before using the apparatus.
Please also follow instructions of the tanks / electrophoresis unit.

Always isolate electrophoresis units from their power supply before removing the safety cover. Isolate the power supply from the mains first then disconnect the leads.
Do not exceed the maximum operating voltage or current (see table 1).
Do not operate the capillary / electrophoresis units in metal trays.
Do not fill the unit with running buffer above the maximum fill lines.
Do not move the unit when it is running.

Acrylamide is a volatile, concentrated neurotoxin which is suspected to be carcinogenic. Please always wear protective clothing when working with acrylamide and follow and observe the working instructions / directions for disposal carefully. Polymerized gels contain residue of unpolymerized monomer. Please wear always protective gloves while working.

Caution:
During electrophoresis very low quantities of various gases are produced at the electrodes. The type of gas produced depends on the composition of the buffer employed. To disperse these gases make sure that the apparatus is run in a well ventilated area.

B. General Care and Maintenance

Clean the apparatus with destilled water only.
Important: Acrylic plastic is not resistant to aromatic or halogenated hydrocarbons, ketones, esters, alcohols (over 25 %) and acids (over 25 %), they will cause „crazing“ especially of the UV transparent plastic and should not be used for cleaning. Do not use abrasive creams or scourers.

Dry components with clean tissues prior to use, e.g. ROTH tissues (ref. 0087.1)

The replacement platinum electrodes are partially shrouded for protection. However, when cleaning the main tank do not use cleaning brushes in the electrode area.
Usually a thorough rinse with distilled water is all that is required.

Ensure that the connectors are clean and dry before usage or storage.

C. Capillary Tube Preparation

- Clean the outside of the tubes in a mild laboratory detergent. A piece of wire with a cotton wool plug placed over the end can be used to clean the insides of the tubes.
- **DO NOT** use abrasive creams or scourers. **NEVER** allow organic solvents or chromic acid to come into contact with the plastic components.
- Handle clean tubes with gloved hands (remove any fingerprints with acetone).

IMPORTANT: Lubricate the Capillary Tubes and Internal Running Module slots with distilled water or running buffer before inserting the tubes.

D. Pouring of Capillary Gels

Ampholyte Gel Solution

7.8 ml	distilled water
1.2 ml	40 % ampholyte solution of the pH range required (see H. Further reagents)
2.52 ml	Rotiphorese Gel 40 (37.5:1)
13.11 g	Urea (to prepare 9 M solution)
2.43 ml	20 % Triton X-100
15 µl	TEMED
150 µl	10 % (v/v) Ammonium Persulphate

D.1. Sealing Method

1. Insert a thin nylon thread inside of the capillary. Be sure that it protrudes on every side for several centimeters.
2. Seal one end of each glass capillary with Parafilm® and place upright in the running module. Insert the glass capillary from the bottom upwards to prevent the Parafilm® getting dislodged.
3. Prepare the following gel solution, using the ampholyte corresponding to the desired pH gradient. There is sufficient volume for twenty 8 cm or ten 17 cm capillary gels.
4. Fill a 10 ml syringe, attached to a 24ga needle, with gel solution. Insert the needle into the glass capillary until it is 1 cm from the bottom. Fill the capillary with gel solution, slowly withdrawing the needle as the fluid level rises. Stop once the gel solution rises to within 1 cm of the top of the capillary.
5. Overlay the top of the gel solution with water-saturated isobutanol. The 1 cm gap will serve as a loading chamber for the sample. Allow the gel solution to set for at least 30 min.

D.2. Capillary Method

1. If capillary action is the preferred pouring method, prepare double the amount of acrylamide solution to that in the recipe above. Pipette ~70 % of the acrylamide solution into a flat-bottomed beaker and stand the capillary tubes upright in the acrylamide solution. Allow the tubes to equilibrate with the acrylamide solution. Check the height of the acrylamide in the tubes. If the tubes are full so that there is less than a 1 cm nonfilled space at the top, remove some of the acrylamide solution from the beaker until the height is 1 cm from the top. If there is a greater than1cm space at the top, add more acrylamide solution, so that the solution rises in the tubes until there is a 1cm space at the top. Fill the 1 cm space, which will act as the sample well, with water saturated isobutanol.
2. Leave to polymerise which will normally take 1-2 hours.
3. After polymerisation, remove the water-saturated isobutanol.
4. The tube gels can now be removed from the beaker and inserted into the slots in the Internal Running Module with the 1 cm sample well facing upwards. Alternatively, they can be stored wrapped in a damp paper towel and Clingfilm at 4 °C. The tubes may contain a residue of acrylamide on the outside and may need cleaning with distilled water before insertion.

E. 1st Dimension (IEF) Phase

Buffer and run conditions will vary according to the type of ampholyte used. The following conditions are given as guidelines only and apply when a pH gradient of approx. 4-8 is the ampholyte used. Other ampholytes will require different buffer solutions. Please consult your laboratory manuals.

Sample Overlay Solution

4.8 g	urea
0.5 ml	40 % ampholyte solution of the pH range required

distilled water to 40 ml

Sample Buffer

5.4 g	urea
1 ml	40 % ampholyte solution of the pH range required
2 ml	20 % Triton X-100
0.1 g	dithiothreitol (DTT)

distilled water to 10ml

1. Fill the tank with low pH anode solution (0.1 % phosphoric acid) according to amounts given in table 2. The buffer has to reach the white plastic ridge, since the lower electrode is located here.
2. Insert the blanking ports into the grommets, if using fewer than 10 capillary gels in the running module. Remove the Parafilm® from the bottom of each glass capillary and insert the capillary running module into the tank. Fill the inner chamber with high pH cathode solution (20 mM NaOH, see table 2), ensuring that the top of each capillary is immersed completely.
3. Add 5 µl of sample overlay solution (see above) to the top of each gel, before replacing the lid.
4. Perform the prerun (pre-focusing) step for 30 min at 200 V (see table 2). The prerun stage is recommended as it helps set up the pH gradient.
5. Replace the safety lid firmly making sure that the electrical connectors form a good contact. The current will gradually decline as the pH gradient forms. After 30 min stop the power supply, turn off the mains supply and disconnect the power leads.
6. Load 2 to 10 µl of sample, previously prepared in sample buffer (see above), with a syringe into the top of each gel. The sample should sink to the bottom of the well.
7. Perform the separation step at 400 V for the time period suggested in the table 2, depending on the length of the capillary gel. Meanwhile, prepare a 12.5 % polyacrylamide resolving gel using a preparatory comb with one large slot (see Info brochure “Polyacrylamide Gels, technical tipps and general considerations”). A gradient gel can be used to resolve proteins of highly contrasting molecular weights.

F. At the End of the Run

1. Perform the ‘End of Run’ stage as suggested in table 1.
2. Turn the power supply settings to zero, turn off the mains supply and **disconnect the power leads.**
3. Turn off the water supply if the unit is being cooled.
4. Remove the safety lid by pressing against the tank from the top and take out the lid by the handles.
5. Remove the capillary module from the tank and carefully remove the capillaries.
6. Use the nylon thread to draw the gel gently out of the capillary. Let it slide onto the gel extraction platform and cut off the thread.
   Alternatively: Using 24ga needle, gently squirt water into the top of the glass capillary so that the gel is extruded onto the gel extraction platform.
   For 2nd dimension runs see G. 2-D Size Determination Phase.
7. Tubes can be cleaned using a mild detergent and rinsing in distilled water. A clean sheet of foam rubber placed at the bottom of the sink serves as a tube support and minimises the risk of tube damage.
8. Empty the tank buffer chambers with a vacuum line and trap or carefully decant the buffer away from the electrical connectors. Rinse the chambers with distilled water then dry the electrode connectors with tissue. Ensure that the connectors are clean and dry before usage or storage.

G. 2-D, Size Determination Phase, 2nd dimension gel

1. Equilibrate the capillary gel for 30 mins. in the running buffer to be used for 2nd dimension.
2. For 2nd dimension we recommend to use 1.5 mm gels. Remove the gel from the buffer solution and align the gel extraction platform with the top of the SDS-PAGE. Allow the gel to slide gently onto the top of the SDS-PAGE gel, making sure that it is in contact with the resolving gel over its entire length.The gel should be casted using a blank or 2-D comb. For details on the casting of slab gels and the use of the Roth Vertical Electrophoresis Units, see the Info brochure “Polyacrylamide Gels, technical tipps and general considerations” and the manual accompanying the vertical electrophoresis chambers.
3. Load the desired protein markers for analysis. Pipette molten 0.5 % agarose with 0.04 % Bromophenol Blue over the tube gel, allowing it to set.
4. Electrophorese as usual for slab gels until the tracker dye has advanced the required distance down the gel.
5. The samples can be visualized using any of the standard staining methods or can be blotted.

H. Further reagents

Ampholytes	pH range	Conductivity (mS/cm)	pH lower limit	pH upper limit	Art. No.
Roti®lyte pH 3-5	3-5	0,1-0,3	3,0±0,5	5,1±0,5	5834
Roti®lyte pH 3-6	3-6	0,05-0,5	3,3±0,3	6,2±0,5	5835
Roti®lyte pH 3-7	3-7	0,2-0,7	3,3±0,3	7,0±0,5	5839
Roti®lyte pH 3-10	3-10	0,2-0,9	3,3±0,3	10,0±0,6	5841
Roti®lyte pH 4-6	4-6	0,1-0,2	4,0±0,5	6,1±0,5	5845
Roti®lyte pH 4-7	4-7	0,1-0,2	4,0±0,2	7,0±0,2	5850
Roti®lyte pH 5-6	5-6	0,05-0,5	5,3±0,3	6,2±0,5	5851
Roti®lyte pH 5-7	5-7	0,1-0,2	5,0±0,2	7,0±0,2	5856
Roti®lyte pH 5-8	5-8	0,1-0,5	5,0±0,5	8,0±0,6	5857
Roti®lyte pH 6-7	6-7	0,1-0,2	6,0±0,2	7,0±0,2	5858
Roti®lyte pH 6-8	6-8	0,15-0,55	6,2±0,5	8,2±0,6	5860
Roti®lyte pH 7-9	7-9	0,2-0,9	7,0±0,3	9,0±0,3	5861
Roti®lyte pH 8-10	8-10	0,1-0,3	7,5±0,5	9,5±0,6	5864

Acetone, >99.5%, for synthesis	5025
Ammonium persulfate (APS)	9592
Bromophenol Blue	A512
DTT	6908
Filter papers	e.g. CL67
Glycerol	3783 or 7530
Isobutanol	6772
Levelling table	N854
NaOH (32 %)	T196
Phosphoric acid	6366
Roti®-Green fluorescent staining solution	1000
Roti®-Load 1, 6 x (reducing)	K929
Roti®-Load 2, 6 x (non-reducing)	K930
Roti®-Red fluorescent staining solution	1045
Rotiphorese® 10 x SDS PAGE running buffer	3060
Rotiphorese®-Blue	A152
Rotiphorese®-Gel 40 (37.5:1)	T802
Roti-Stock 20 % SDS Solution ready-to-use	1057
SDS	2326
SDS Pellets	CN30
Silver staining Roti®-Black P (Protein)	L533
Silver staining/Bleaching Roti®-Bleach	N766
TEMED	2367
Tris	AE15
Tris-Cl prebuffered	9090
Triton X-100	3051
Urea	2317
Water double distilled	3478
Xylene cyanol	A513

Carl Roth GmbH+Co. KG
Schoemperlenstraße 3-5
76185 Karlsruhe
Postfach 100121
76231 Karlsruhe

Telefon: (+49)721/5606-0
Telefax: (+49)721/5606-149
E-Mail: info@carlroth.de
Internet: www.carlroth.de

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For further data and safety recommendations please see our catalogue or the internet at carlroth.de or carlroth.com